Genetics and Biochemistry in Neurospora

In spite of the title, this talk is in no way an attempt to mimic or duplicate the lectures such as the Harvey Society lectures that have been given by the godfather of all this work, Dr. GW Beadle, but will deal for the most part with observations made in this laboratory.

The work that I am about to describe arose in my mind out of Avery's classical work on Pneumococcus transformation. As my teachers and friends will no doubt recall from my undergraduate days at Columbia, the very sight of a Drosophila was repulsive in my eyes, and I would no more have thought of counting fruitflies than of isolating spores of some unimportant bread mold called Neurospora. The Pneumococcus transformation was however a biologic fact of great potential importance in medical bacteriology, and it seemed worthwhile looking at it. However, it seemed desirable to attempt to duplicate such an experiment in an organism whose genes were located in chromosomes, in the hope of finding out more about it. Such an organism was Neurospora crassa, in which Beadle and his school had of course done such a remarkable bit of work. I took this crackpot, and by no means novel idea to Ryan, and discovered to my amazement (and I hope he will not object to my revealing this) that he had planned much the same experiment: an attempt to convert nutritional mutants to the wild type using killed extracts of wild type mycelium. I have never spent a more pleasant vacation week on an experiment that did not work. The results were encouraging at first, but it soon appeared that as many cultures were being converted to a 'wild type' in the untreated control series, as in the series which were subjected to extracts of Neurospora mycelium. Transformation is therefore still an open question; it was necessary first to study the spon-

taneous reversion. It might be added parenthetically that attempts of a similar nature on non-reverting nutritional mutants of E. coli have failed miserably.

An examination of the literature showed that 'adaptation' had been described before in several mutants; indeed the 'adaptation' of the leucinless strain was one of the most difficult bugs of the assay method for leucine described by Ryan and Brand. The phenomenon has apparently never been studied in detail however. Briefly it was found that several mutants which while they would not grow initially on inoculation into a medium deficient in their grwoth factor requirement, occasionally would grow up on prolonged incubation. In many cases, conidia taken from such an adapted culture would, on inoculation into fresh minimal medium, show the immediate grwoth characteristic of the wild strain; in some instances they would not, and it is apparently his study of such a strain (16117: isoleucine-valineless) that had led Beadle to the conclusion that adaptation did not involve a cahnge in the genetic constitution of the strain. For our first studies it seemed advisable to examine cases of the first type, and of these our attention has been directed primarily at the leucineless and the pabless mutants,

A brief review of the biochemical genetics of Neurospora may help to clarify what follows. Neurospora crassa is an ascomycetous mold. The 8 spores in the ascus, as Dr. McClintock has explained to this samecolloquium in the past are so disposed that each pair, barring slippage is genetically identical, and the four pairs represent the haploid segregants of the four chromatids at meiosis. The mycelium contains haploid nuclei, but there are a great many of them in each cell, and the cross walls have perforations large enough for the nuclei to pass thrpugh, so that the entire mycelium is syncytial. The haploid condition of the mycelium is rigorously maintained, so far as is known, until zygote formation takes place. There are two well defined mating types, defined by a single gene, so that Neurospora is an obligate heterothall,

The tetrasperma species of Neurospora differ insofar as they mainatin a mechanism whereby the ascus' four spores are each bicaryotic and contain 2 nuclei of opposite mating type. The diploid fusion nucleus (F-1) is reduced in 2 meiotic divisions, as in other forms, and the haploid segregants undergo a further 2 mitotic divisions, so that each member of a pair of spores contains 2 identical haploid nuclei.

By X-raying or UV-raying conidia or spores, Beadle has obtained a variety of mutations. These are manifest in the f-1, since in the haploid organism dominance is not a factor.

after Craig: The Laboratory Diagnosis of Protozoan Diseases and original observations.

Feature and stage.	Benign Tertian Mal. Plasmodium vivax	Malignant Tertian Mal. Plasmodium falciparum	Quartan Mal. P. malariae	Remarks.
Ring stage and early trophozoite	Usually very symmetrical in earliest stages. May show signs of amoeboid activity 1/3 - 1/2 diam. of RBC. *	1/6 - 1/4 diam. Slender ring. Presence of 10% or more of RBC with 2 or more rings highly suspicious. Maurer's dots. Heavy infection with 4 rings.	Scim. to P. vivax 2 rings not found. Usually rather light infection.	Positive diagnosis very difficult at this stage. *
Trophozoite	Irregular in shape. Extensive, light pigment Enlargement of RBC Hypochromia RBC Schuffner's dots. †	Not found in peripheral blood.	Regular, occ. elongated shape (Equatorial or band form) ‡ No enlargement or hypochromia. pigment darker.	A clear and marked cremation and oval shape of RBC, with heavy pigment and granulation of cytoplasm, especially in patients from W. Africa or China suggests P. ovale the agent of "ovale tertian malaria" It is quite rare.
Schizont.	More regular than troph. as above. Schizogony more rapid than P. malariae	"	As above.	
Merocyte	12 - 20 or more merozoites, usually 16. Light pigment, eccentric aggregation. Merozoites not regularly arranged. Enlargement.	" 8 - 10.	6 - 10 merozoites Rosette forms. central dark pigment. No appreciable enlarge.	
Gametocyte	Slightly amoeboid. Enlargement and hypochromia.	Crescent and sausage-shaped forms.	Regularly shaped dark pigment. No enlargement!	

* Some cases of P. vivax show a small number of RBC with 2 rings, very rarely more. P. falciparum not uncommonly shows 4-5 rings. Early therapy may make the diagnosis of P. falciparum very uncertain. The presence of rings with 2 chromatin dots occurs also in P. vivax, but is usually characteristic of P. falciparum. "Appliqué forms" are more frequent in, but not diagnostic of P. falciparum.

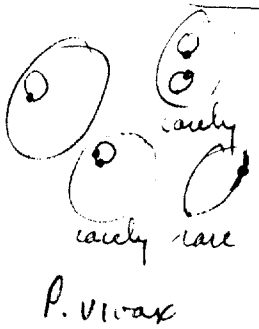
† Schuffner's dots usually appear best in Wright's stain. They should not be used as a diagnostic feature.

‡ Band forms rarely occur in P. vivax. The lack of enlargement of RBC is diagnostic.

differential diagnosis has recently been made on the patient.

The following sketches summary: (exaggeratedly)

Ring.

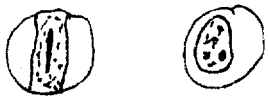


P. vivax

P. malariae

P. falciparum.

Trophozoite.



Band form. Round form.

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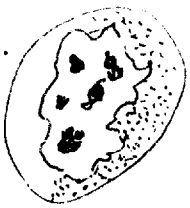
P. ovale

Schizont.



Merocyte

Gametocyte.